FORM PTO-1390 (REV 10-94) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED .OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		01-492
		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/913545
INTERNATIONAL APPLICATION NO. PCT/EP00/01214	INTERNATIONAL FILING DATE February 15, 2000	PRIORITY DATE CLAIMED February 17, 1999

TITLE OF INVENTION
METHOD REPRESENTING BIOLOGICALLY ACTIVATED INDUCTANCE-ALTERING PARTICLES AND DEVICE...

APPLICANT(S) FOR DO/EO/US
Kilian Hennes

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☒ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☐ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:

Applicant claims small entity.

(January 1995)

Express Mail No.:
EL394335879US

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on August 15, 2001
(Date of Deposit)

Rachel Piscitelli
Name and Reg. No. of Attorney

Rachel Piscitelli
Signature

August 15, 2001
Date of Signature

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 09/913545	INTERNATIONAL APPLICATION NO. PCT/EP00/01214	ATTORNEY'S DOCKET NUMBER 01-492

17. ☒ The following fees are submitted:	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO..... $860.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482)		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2))...		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$1,000.00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4).....		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00	
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	14 -20 =		X $18	$	
Independent claims	1 -3 =		X $80	$	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+$270	$	
TOTAL OF ABOVE CALCULATIONS =				$ 990.00	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28).				$ 495.00	
SUBTOTAL =				$ 495.00	
Processing fee of $130.00 for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 130.00	
TOTAL NATIONAL FEE =				$ 625.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$	
TOTAL FEES ENCLOSED =				$ 625.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 625.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-0184. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SIGNATURE

Gregory P. LaPointe

NAME

28,395

REGISTRATION NUMBER

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on August 15, 2001
(Date of Deposit)

Rachel Piscitelli
Name and Reg. No. of Attorney

Rachel Piscitelli
Signature

August 15, 2001
Date of Signature

H264WP3

DESCRIPTION

Method of representing biologically activated inductance-altering particles and device for carrying out the method

The invention concerns a method of representing biologically activated inductance-altering – in particular ferromagnetic or superparamagnetic – particles. The invention further concerns a device for detecting and counting suspended biological microparticles in liquid samples, in particular for carrying out the specified method.

Hitherto the procedure involved in counting bacteria, blood cells or cell constituents in aqueous solutions has been effected by means of through-flow cytometers or Coulter counters. Here the corresponding particles are colored and identified on the basis of optical signals or counted by capacitive measurement procedures.

In consideration of those factors the inventor set himself the aim of simplifying such measurement operations.

That object is attained by the teaching of the independent claim; the appendant claims set forth advantageous developments. In addition the scope of the invention embraces all combinations comprising at least two of the features disclosed in the description, the drawing and/or the claims.

In accordance with the invention monovalent primary antibodies are mixed with inductance-altering, in particular ferromagnetic or superparamagnetic, particles in multiple excess, which are coated with secondary antibodies; aggregated particles which comprise a monovalent primary antibody and antibody-coated ferromagnetic partial particles are then separated by means of partial sedimentation in a centrifuge. Instead of primary antibodies it is also possible to use viruses or gene samples,

whose sheathing proteins or spacer molecules are targeted by the secondary antibodies.

In accordance with a further feature of the invention the detecting or counting biological particles are immunologically, phagologically or molecular-biologically joined to aggregated particles which, when subsequently flowing through a metal coil – in particular the gap of a C-shaped metal coil with a ferromagnetic core – trigger measurable and countable alterations in inductance.

It has also proven to be advantageous for inductance-altering particles, before flowing through the metal coil, to be retained by means of an electromagnet in a plastic capillary and there to be joined to the biological particles flowing into the capillary, while the sample in which same were contained is taken out of the capillary. In addition, countable alterations in the natural oscillation frequency are to be produced by the metal coil as part of an electronic resonant circuit.

In order to obviate the apparatus expenditure in regard to optical measurement and to achieve a higher degree of specificity in comparison with capacitive measurement, a different measurement principle is therefore used for detection of the individual particle: measurement of the alteration in inductance of a microcoil of metal. As however biological particles have a permeability constant μ of approximately 1, they have to be previously marked by means of inductance-altering substances for detection and counting procedures by means of a coil. That marking is effected by immunological, phagological or molecular-biological coupling of ferromagnetic or superparamagnetic particles which are monovalently joined either to antibodies, virus docking molecules or gene samples at spacer molecules.

The scope of the invention includes a device of the kind set forth above, having a delivery line for a sample to be measured, which is surrounded as a measuring line by a metal coil as a measuring coil which

in turn is connected to a device for exciting oscillation and measuring resonance events.

In a particular embodiment that metal coil is laid around a core which is bent approximately into a C-shape and whose ends delimit a gap; the measuring line is laid through that gap.

In accordance with a further feature of the invention the delivery line is connected to a device with capillaries – in particular with Teflon capillaries - ; the latter are associated with an electromagnet and can be arranged in a space surrounded by a pole piece.

Advantageously provided between the electromagnets and a valve of the delivery line is a branch line for excess sample. In addition at least one resistor and a capacitor can be arranged in front of each device for exciting the oscillations and measuring resonance events, towards the metal coil.

The measuring coil, a piezoelectric pump arranged upstream thereof and a downstream-arranged resistor and capacitor respectively are to be parts of a microsystem-technical unit in accordance with the invention.

Therefore coupling of the ferromagnetic markers occurs in the device which at the same time permits enrichment of the particles to be counted: the markers are retained in the Teflon capillary by means of an electromagnet as a sorption layer, until the entire sample has been pumped into the capillary and at the same time the excess sample has run out of the capillary. Thereupon the magnet is switched off so that the markers freely diffuse and can saturate the surface of the biological particles. The capillary content is then pumped by the above-mentioned piezoelectric pump through the metal coil, in particular through the gap of the metal coil, which is of a C-shaped configuration, with a ferromagnetic core. The metal coil is etched in the form of a spiral onto a circuit board and is connected with capacitor and resistor as a resonant circuit. The resonant circuit is excited by a frequency corresponding to that natural resonant frequency which is generated when an averagely marked

biological microparticle is in the gap or the coil. As a result a resonance oscillation always occurs in the resonant circuit when a corresponding microparticle passes through the coil.

An example of the use of that method is the detection of coli bacteria in water samples. For that purpose monovalent primary E.-coli-specific antibodies are conjugated with secondary antibodies coupled to magnetic beads. The suspension of those conjugates is pumped into the Teflon capillary and fixed there by means of an electromagnet. When the water sample to be investigated flows through the capillary, coli bacteria are retained to the conjugates by way of the primary antibodies. After the magnet is switched off the suspension of magnetically marked coli bacteria can be pumped through the measuring coil or the gap of the metal coil. The number of resonance events in the connected resonant circuit corresponds to the number of coli bacteria in the original water sample. By virtue of the use of that arrangement and the corresponding conjugates, it is possible to automatically count bacteria without the expensive use of through-flow cytometry. Furthermore it is possible with that measuring method to achieve miniaturization of the detection arrangement.

The described procedure is used for detecting and counting particles such as bacteria, cells or cell constituents in aqueous solutions. That procedure permits miniaturization of the automatic particle counting method. For that purpose the particles are marked prior to the measurement procedure by the reaction with monovalent antibody-coated or virus-coated ferromagnetic particles. Inductive measurement is based on passage of the ferromagnetic particles aggregated with the biological particles through the microcoil, designed in the above-described manner, of an electronic resonant circuit. The resonance events which occur upon such particle passage are counted.

The device according to the invention can be used in medicine, microbiology and hygiene, for example for counting out blood cells; it is

possible to count out ecologically relevant micro-organisms or detect pathogenic germs.

Further advantages, features and details of the invention will be apparent from the description hereinafter of a preferred embodiment and with reference to the drawing in which:

Figures 1 and 3 each show a diagrammatic view relating to a method according to the invention, and

Figure 2 is a diagrammatic perspective view of a detail from Figures 1 and 3.

Prior to a method of detecting coli bacteria in a water sample Z supplied through a line 10 monovalent primary E.-coli-specific antibodies are conjugated to secondary antibodies coupled to magnetic beads. The line for the monovalent magnetic particles F is denoted by reference 12. Both lines 10, 12 include hose pumps 14 and downstream of same are combined to form a common delivery line 16.

The reagent with ferromagnetic, biologically activated particles is pumped by way of the lines 12 and 16 into a Teflon capillary 20 and is fixed there by means of an electromagnet 22 whose magnetic coil is identified by reference numeral 24 and with which there is associated the Teflon capillary 20 which is wound on in a z-shape, in a concentric pole piece 26. The latter with a pole pin 28 surrounded thereby at a radial spacing defines an annular space 30 for the Teflon capillary.

When the water sample Z to be investigated flows through the capillary 20 coli bacteria as biological particles to be counted are retained by way of the primary antibodies to the ferromagnetic conjugates. After the electromagnet 22 is switched off the suspension of magnetically marked coli bacteria can be transported by virtue of a piezoelectric pump 32 in a measuring line 34 through an etched metal coil as a measuring coil 36 of a microsystem-technical unit 40. The counted particles are discharged therefrom in the direction indicated by the arrow X.

In the embodiment of Figure 3 the suspension is transported in the measuring line 35 through the gap 52 of a ferromagnetic core 50 of a measuring coil 36_a, the core 50 being curved in a C-shape.

The free ends 38, 38_a of the measuring coil 36, 36_a – downstream of a resistor 42 and a capacitor 44 – are connected to a device 46 for exciting the oscillation and for measuring resonance events; there conversion into counting pulses takes place.

The number of resonance events in the connected resonant circuit corresponds to the number of coli bacteria in the original water sample Z.

Provided between the Teflon capillary 20 and the piezoelectric pump 32 is a line branch 18 – which includes a valve 48 – for excess sample portions Q, with a valve 48 being connected downstream thereof in the delivery line 16.

CLAIMS

1. A method of representing biologically activated inductance-altering, in particular ferromagnetic or superparamagnetic, particles, characterized in that monovalent primary antibodies are mixed with inductance-altering particles in excess, which are coated with secondary antibodies, and then aggregated particles which comprise a monovalent primary antibody and antibody-coated inductance-altering partial particles are separated by means of partial sedimentation.

2. A method of representing biologically activated inductance-altering, in particular ferromagnetic or superparamagnetic, particles, characterized in that viruses are mixed with inductance-altering particles in excess, which are coated with antibodies targeting the sheathing proteins of the viruses, and then aggregated particles which comprise a virus and antibody-coated inductance-altering partial particles are separated by means of partial sedimentation.

3. A method of representing biologically activated inductance-altering, in particular ferromagnetic or superparamagnetic, particles, characterized in that spacer molecule-coupled oligonucleotide gene samples are mixed with inductance-altering particles in excess, which are coated with antibodies targeting the spacer molecules, and then aggregated particles which comprise a gene sample and antibody-coated inductance-altering partial particles are separated by means of partial sedimentation.

4. A method as set forth in one of claims 1 through 3 characterized in that biological detection or counting particles are immunologically, phagologically or molecular-biologically combined with the aggregated particles which as markers when subsequently flowing

through a metal coil trigger off measurable and countable alterations in inductance.

5. A method as set forth in claim 4 characterized in that when flowing through the gap at a core, which is curved substantially in a C-shape, of a metal coil the markers trigger off measurable and countable alterations in inductance.

6. A method as set forth in claim 4 or claim 5 characterized in that inductance-altering particles are retained prior to flowing through the metal coil by means of an electromagnet in a plastic capillary and are combined there with the biological particles flowing into the capillary while the sample containing same is passed out of the capillary.

7. A method as set forth in one of claims 4 through 6 characterized in that countable alterations in the natural resonant frequency are produced by the metal coil as part of an electronic resonant circuit when the inductance-altering particles flow therethrough.

8. A device for detecting and counting suspended biological particles in liquid samples, in particular a device for carrying out the method as set forth in at least one of the preceding claims, characterized in that a delivery line (16) for a sample to be measured is surrounded as a measuring line (34) by a metal coil as a measuring coil (36, 36_a) and same is connected to a device (46) for exciting oscillation and measuring resonance events.

9. A device as set forth in claim 8 characterized in that the metal coil (36_a) is laid around a core (50) which is curved substantially in a C-shaped configuration and the core has a gap (52) through which the measuring line (34) is passed.

10. A device as set forth in claim 8 or claim 9 characterized in that the delivery line (16) is connected to a device with capillaries (20), in particular Teflon capillaries, and the latter are associated with an electromagnet (22).

11. A device as set forth in claim 10 characterized in that the capillary or capillaries (20) are arranged in a space (30) surrounded by a pole piece (24).

12. A device as set forth in one of claims 8 through 11 characterized in that arranged between the electromagnet (22) and a valve (48) of the delivery line (16) is a branch line (18) for excess samples (Q).

13. A device as set forth in one of claims 8 through 12 characterized in that arranged upstream of the device (46) for exciting the resonance and measuring resonance events towards the metal coil (36, 36_a) are at least one resistor (42) and a capacitor (44).

14. A device as set forth in one of claims 8 through 13 characterized in that the measuring coil (36, 36_a) with upstream-arranged piezoelectric pump (32) and downstream-arranged resistor (42) and capacitor (44) respectively are parts of a microsystem-technical unit (40).

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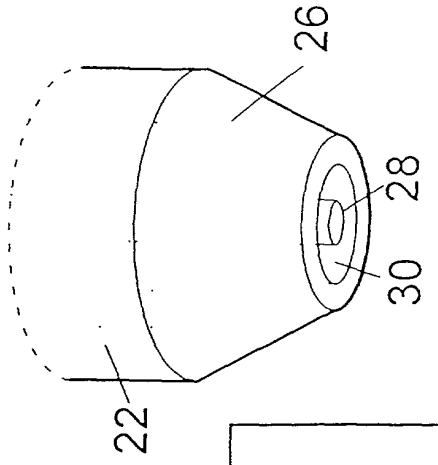
Amended new claims (11.06.2001)

8. A device for carrying out the method as set forth in one of claims 1 - 7, for detecting a biological particle which is conveyed through a conveyor line and which is bonded to a marking particle of inductance-altering, in particular ferromagnetic or superparamagnetic material, wherein the delivery line (16) for a sample to be measured is surrounded as a measuring line (34) by a metal coil as a measuring coil (36, 36_a) and same is connected to a device (46) for exciting oscillation and measuring resonance events, and wherein the metal coil (36_a) is laid around a core (50) which is curved substantially in a C-shaped configuration and the core has a gap (52) through which the measuring line (34) is passed.

9. A device as set forth in claim 8 characterized in that the marking particle is monovalently bonded to at least one biological particle.

Fig. 2


26
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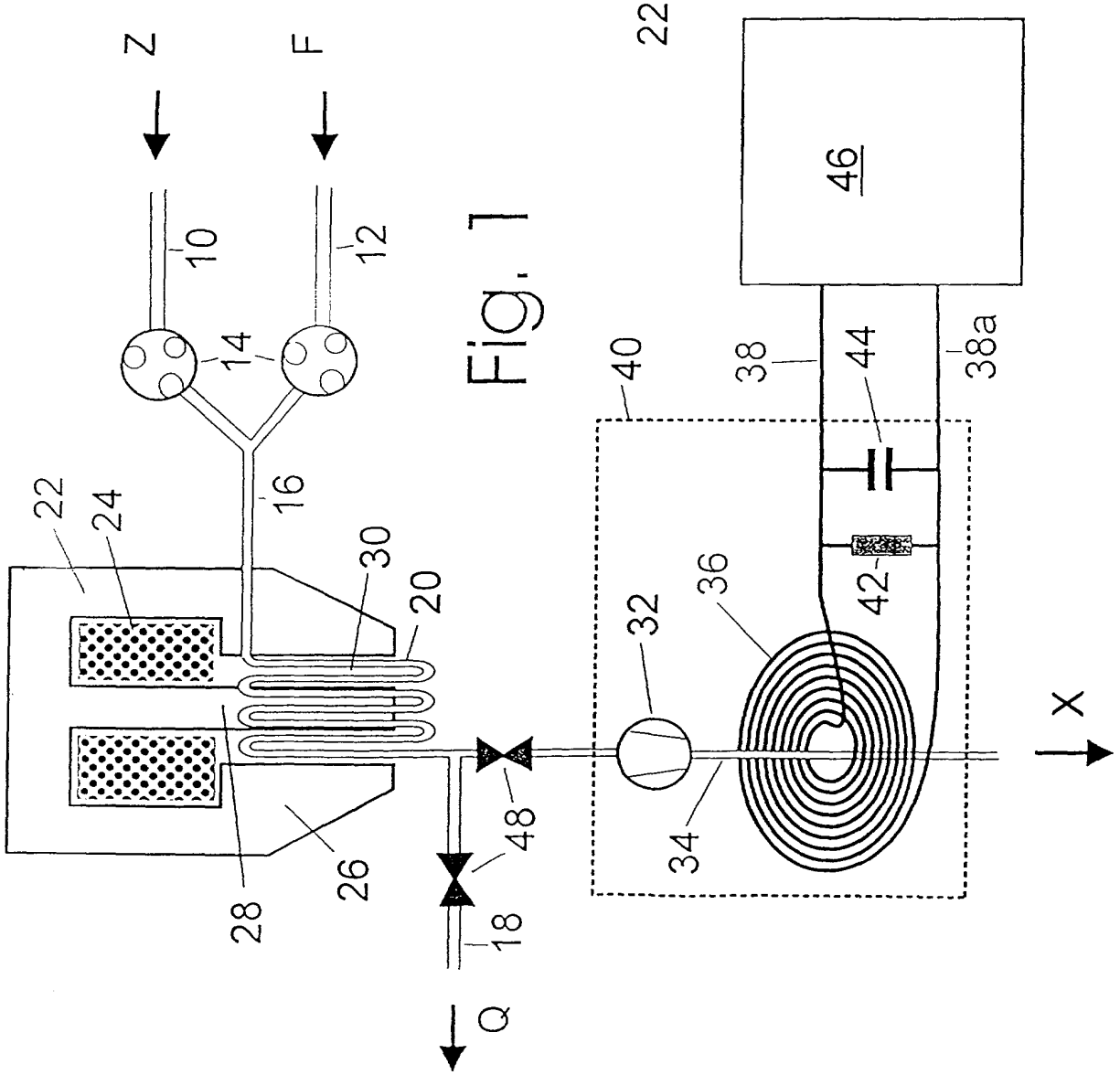
22

Fig. 1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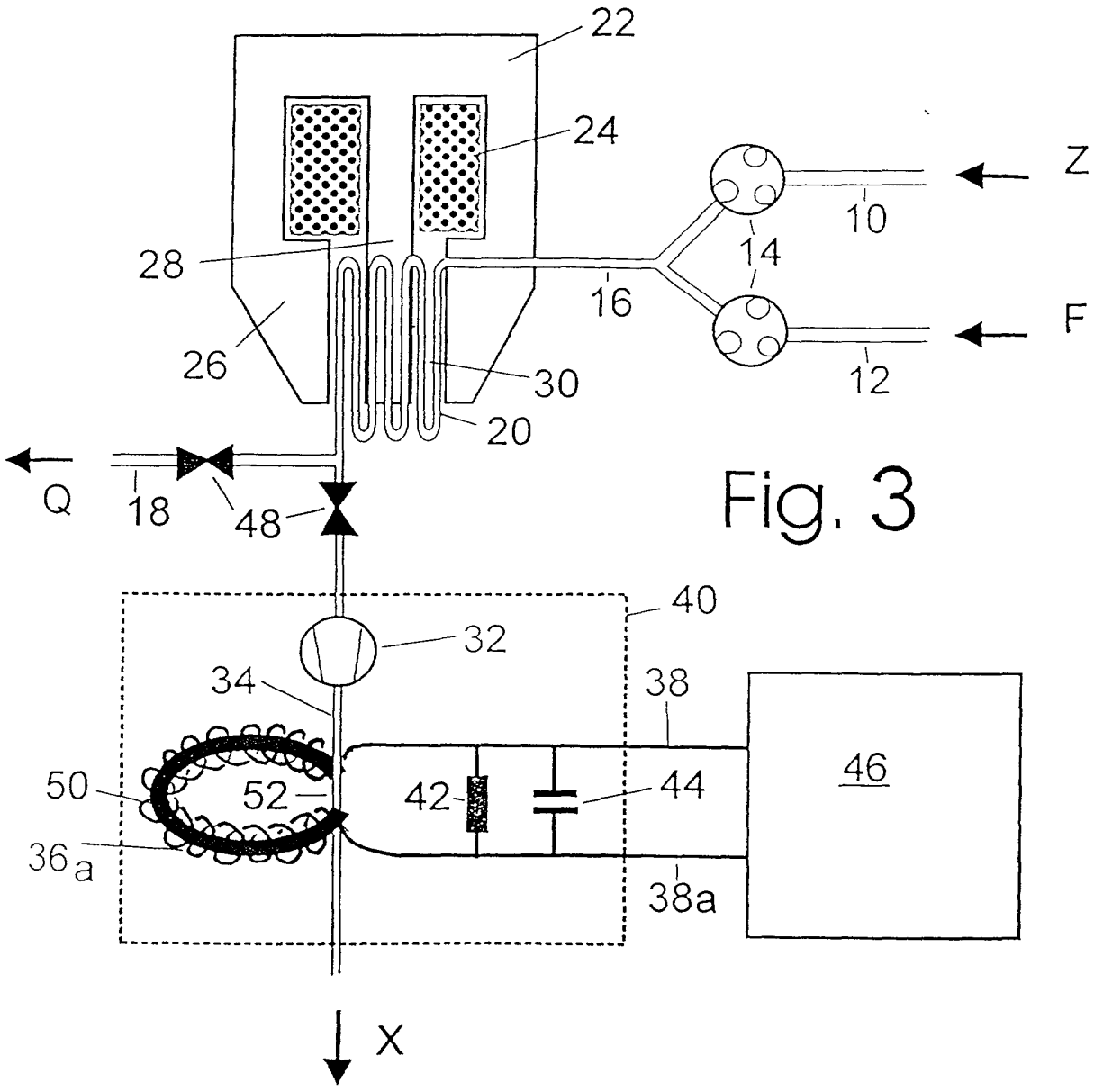
22
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32
34
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46
50
52
42
44
36a
38a
X

Fig. 3

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Method for Representing Biologiacally Activated inductance-Altering Particles and Device for Carrying out the Method

Case No. 01-492, the specification of which

(check one)

_____ is attached hereto.
XXX was filed on August 15, 2001 as Application Serial No. 09/913,545
~~xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx~~ or PCT/EP00/01214 filed August 15, 2000.
~~(if applicable)~~

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a) and understand that information is material where there is substantial likelihood that a reasonable examiner would consider it important in deciding whether to allow the application to issue as a patent.

I do not know and do not believe this invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as identified below:

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below

Prior Foreign Application(s)

Number	Country	Date
199 06 352.4	Germany	Eebruary 17, 1999
199 39 208.0	Germany	August 18, 1999

and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the above listed application on which priority is claimed:

Prior Foreign Application(s)

Number	Country	Date

If no priority is claimed, I have identified all foreign patent applications filed prior to this application:

Prior Foreign Application(s)

Number	Country	Date

And I hereby appoint Robert H. Bachman (19,374), Gregory P. LaPointe (28,395), Richard S. Strickler (18,228), and Barry L. Kelmachter (29,999), all members of the firm of Bachman & LaPointe, P.C., A Professional Corporation.

Gregory P. LaPointe
Telephone: 203-777-6628

my attorneys with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and direct that all correspondence be forwarded to:

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900 Chapel Street
New Haven, CT 06510-2802

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor HENNES, Kilian

Inventor's signature _____ Date 14.8.2001

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Citizenship German

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